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REVIEW

New Trends in the Regulation of the Gonadal Activity in Vertebrates: Paracrine and Autocrine Control

Giovanni Chieffi

Istituto di Biologia, I Facoltá di Medicina e Chirurgia, Universitá di Napoli, and Stazione Zoologica, Napoli, Italy

INTRODUCTION

The gonadal function of vertebrates is regulated by an intricate interplay of exogenous and endogenous factors. As far as endogenous factors are concerned, neuroendocrine control of gonadal function has been largely investigated (see for review, Chieffi [1]). However interesting data were accumulating in the recent years in favour of a local control of both gonadal compartments, the germinative and endocrine. The term "ultrashort feed-back" was also used in this case. This term was introduced by Motta and coworkers [2] to explain the modulation of synthesis, storage and release of the hypothalamic releasing hormones by changes of their own titers in the general circulation. Therefore the term "ultrashort feedback" became misleading in case of a local control of gonadal activity, since this type of control does not use systemic circulation. It could be also suspected that part of the ultrashort feedback, as defined by Motta and coworkers [2], might correspond to local mechanisms of control. This hypothesis might be true especially in the case of hypothalamic releasing factors whose half life is known to be very short. Therefore I would suggest to keep the term "ultrashort feedback" for the endocrine autoregulation, i.e. via blood stream as it was first used, and to use the terms "autocrine" and "paracrine" (which are already used by several authors) controls for the local autoregulation.

Autocrine control occurs when one cell is able to regulate its own functions using one of its secretory products. The substance produced within the cell acts through raising receptors and modulating the sensitivity of diverse biochemical processes to stimulation by other hormones (Fig. 1B).

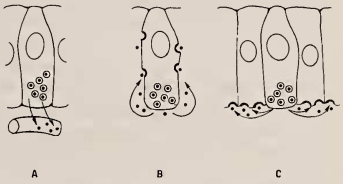

Fig. 1. Various types of cell to cell communications: endocrine (A), autocrine (B), paracrine (C). Chemical messangers are shown in latent form within the cell. Thickened regions of the cell membrane represent receptor sites; however receptors to some hormones may be intracellular.

Various lines of evidence indicate another type of local control as well, the so-called "paracrine control"; it became known as a generalized form of bioregulation whereby one cell in a tissue selectively influences the activity of an adjacent different cell type through the biosynthesis and release of chemical messengers which diffuse into the parenchyma and act specifically on neighbouring target cells. However, it should be remembered that a formal assignement of a paracrine regulatory role requires a substance to be present, locally biosynthesized, and to exert a receptor-mediated biological effect in the tissue in question (Fig. 1C).

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In this review only selected aspects of this kind of regulation of gonadal activity will be taken into consideration. Paracrine and autocrine communications involve locally produced steroidal and nonsteroidal bioregulators. The list of candidates for involvement in gonadal paracrine and autocrine communications is growing rapidly as new factors are identified in the gonads or are shown to have direct effects *in vitro*.

TESTICULAR LOCAL CONTROL

As far as the testis is concerned, as in any organ or tissue containing different cell types, local coordination of the function of different cell types is fundamental to the efficient working of the organ.

Testis is a composite organ, whose specific components offer a valuable model to investigate possible cell to cell communication. Although testicular function drops following gonadotropins withdrawl, the reception, trasmission and/or utilization of these pituitary signals must be locally modulated or mediated. Since heterogeneity of seminiferous tubules and adjacent interstitial cell function exists within the testis, local requirements for testosterone production cannot be satisfied by the circulating LH. Therefore local factors should provide appropriate modulation of androgen production.

Overview of mammals

An important impulse to the knowledge of testicular paracrinology was given by the technique of transillumination-assisted dissection of rat seminiferous tubules devised by Parvinen [3] and Parvinen and Ruokonen [4]. The embracement of the spermatogonia by a Sertori cell from the onset until the end of spermatogenesis led to investigate the intimate exchange of information between the two cell types. The simplest function of physical and nutritional support for the growing spermatogonia attributed to the Sertoli cell turned into a number of impredictable biochemical and metabolic events during spermatogenesis.

Figure 2 summarizes only some of the cyclical changes in Sertoli cell secretion in the rat that Sharpe [5] well defines as "the tip of the iceberg". In fact Sertoli cells secrete certainly numerous proteins; the identity and function are still unknown for most of them.

Stages I-IV of spermatogenetic cycle of the rat are characterized by a high concentration and responsiveness of FSH receptors. At stages IV-VI ceruloplasmin, a copper-transporting protein whose function is still vague, is detected at highest concentrations. At stage VI peaks also a protein of unknown function called "cyclic protein 2", while FSH receptors reach the lowest concentration. Maximal secretion of the androgen binding protein (ABP), along with the highest secretion of an aromatase inhibitor, occurs at stage VII when meiosis commences. Increase of the plasminogen activator, a protease, at stages VII and VIII seems to be related to spermiation, residual body ingestion and meiotic germ cell translocation. Finally, transferrin, an iron-transferring protein, is produced in large amounts from stage IX until XIV. During this period lipid accumulation is also observed in Sertoli cells (see for review, Sharpe [5]).

These are simply the most remarkable cyclic metabolic changes occurring in the Sertoli cell during spermatogenesis of the rat and likely represent the response to germ cell requirements.

It is worthy of notice that co-culture of Sertoli cells with pachytene spermatocytes stimulates Sertoli cells' ABP secretion [6]; however co-culture of Sertoli cells with spermatids does not stimulate ABP secretion [7] thus indicating the possible existence of specific "orders" sent by the germ cells to Sertoli cells at different stages of the spermatogenic cycle; Sertoli cells in turn trigger the spermatogenic events in appropriate succession.

Concomitantly, Sertoli cells interact with peritubular cells. The growth factor, somatomedin-C, present in the peritubular cells, has binding affinity for Sertoli cell receptors [8]. Sertoli cells also interact with Leydig cell directly and/or through peritubular cell mediation. Leydig cells are known to produce, besides testosterone and neurohypophysial-hormones like substance, a number of other hormones such as estrogens, prostaglandins, angiotensin, β-endorphin. According to Drummond *et al.* [9], testosterone regulates inhibin production by Sertoli cells. As regard to the paracrine-autocrine control of AVP-like peptide,

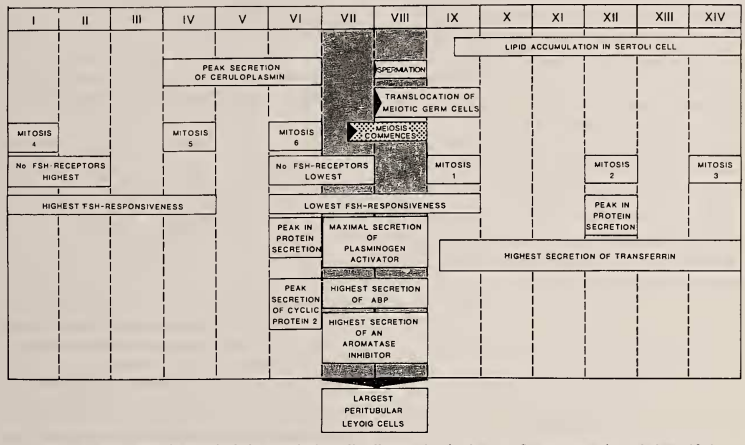


FIG. 2. Scheme of the major cyclical changes in Sertoli cell secretion in the rat. Spermatogenic cycle lasts 12 day and is divided into 14 stages (I-XIV). The shaded area (stages VII and VIII) corresponds to the most androgen dependent period of the spermatogenic cycle (Sharpe [5]).

Kasson *et al.* [10] propose that pituitary LH interacts with specific receptors on Leydig cell membranes activating cholesterol side-chain cleavage enzyme, 17α-hydroxylase and 17, 20-desmolase thus increasing testosterone production. This is locally regulated by an AVP-like peptide which binds to specific receptors on Leydig cells increasing progesterone biosynthesis but decreasing androgen secretion affecting 17α-hydroxylase and 17, 20-desmolase activities (Fig. 3).

Testosterone is the main hormone which acting

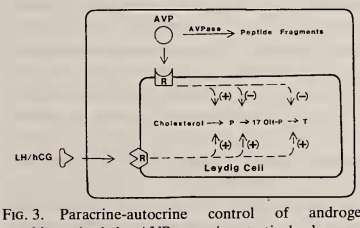


FIG. 3. Paracrine-autocrine control of androgen biosynthesis by AVP as an intratesticular hormone (Kasson *et al.* [10]).

on Sertoli cells drives spermatogenesis. There are many convincing evidences for a local regulation of testosterone secretion by Sertoli cells. The first and the most thoroughly investigated factor, probably produced by Sertoli cells, is a LHRH-like factor. This differs from hypothalamic LHRH and stimulates Leydig cell testosterone production via pathways different from those utilized by LH (see for review, Sharpe [5]). Recently, a number of factors different from LHRH-like substances have been claimed to exert stimulatory effect on rat Leydig cell testosterone production, at least *in vitro* [11–16].

Very recently, Hsueh *et al.* [17] have shown that inhibin related gene products synthesized by Sertoli cells may form heterodimers or homodimers to serve intragonadal paracrine signals in the modulation of LH-stimulated androgen biosynthesis. It has been shown that $\alpha\beta$ heterodimer of inhibin enhances LH-stimulated androgen production by primary cultures of neonatal testicular cells. In contrast $\beta\beta$ homodimer of inhibin suppresses Leydig cell androgen production at all doses of LH tested. When both heterodimer and homodimer

are given simultaneously, an intermediate level of androgen biosynthesis is induced by LH (Fig. 4 A). Mismatching experiments confirmed these results. In fact, increasing doses of heterodimer or homodimer respectively enhance or inhibit LH-stimulated androgen secretion (Fig. 4 B).

This local control modulates the feedback between inhibin and gonadotrops through the suppressing or stimulating effect of gonadotropin secretion by androgen feedback. Therefore the final goal of both control mechanisms (central and local) is to act coordinately in stimulating or suppressing FSH release (Fig. 5).

Very recently Lee *et al.* [18] have shown that $\beta\beta$ homodimer is produced probably by the Leydig cells. If so, $\beta\beta$ homodimer local effect on Leydig cells should be considered of the autocrine type.

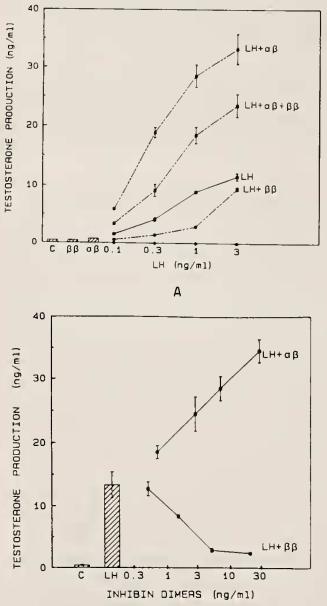


FIG. 4. Modulatory effects of inhibin dimers on LH-stimulated androgen production by cultured neonatal testis cells of the rat. A, Modulatory effect of inhibin dimers on increasing doses of LH. B, Mismatching experiments using increasing doses of inhibin dimers (Hsueh *et al.* [17]).

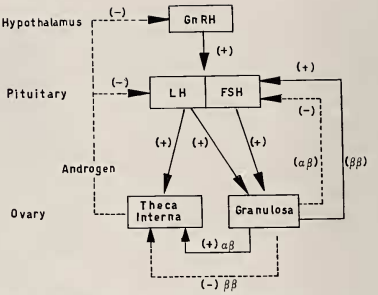


FIG. 5. Diagram of the hypothalamic-pituitary-testicular feedback mechanisms. Based on findings of paracrine and endocrine actions of heterodimer of inhibin subunits, the cross-communication between two hypothalamic-pituitary-testicular feedback axes can be postulated. LH acts exclusively on testis Leydig cells to stimulate the production of androgens that exert negative feedback on LH secretion. Likewise, FSH stimulates the production of Sertoli cell inhibin that suppresses FSH release but enhances the LH stimulation of androgen biosynthesis at the Leydig cell level. In addition, the $\beta\beta$ homodimer may exert pituitary and intratesticular actions opposite to those induced by the inhibin heterodimer (Hsueh *et al.* [17]).

Also testicular vasculature is probably under paracrine control and work is progressing in order to elucidate this important aspect of gonadal function (see for review, Sharpe [5]).

Although rat testis has been the almost unique model so far investigated, the scarse literature referring to other mammlian species, included man, does not speak of significant species differences. In general, the process of spermatogenesis and its dependence on testosterone are very similar in the rat, man and other mammlian species.

As regard to the autocrine control, it becomes difficult in some cases to distinguish it from the paracrine control, owing to the still unknown origin of certain testicular factors. This is the case of the above mentioned AVP-like peptide, which modulates testosterone production by the Leydig cells [10]. Murono and Payne [19] observed inhibi-

tion of the testicular 17β-hydroxysteroid dehydrogenase *in vitro* by androgens in the rat testis. It was then reported by Naessany *et al.* [20] that physiological concentrations of testosterone were ineffective in inducing inhibition of testosterone production by whole testis in organ culture. Using a different experimental model, i.e. the fetal testicular suspension, Pointis *et al.* [21] demonstrated that doses of testosterone and DHT in the same range (20 times greater than that found in the male fetal circulaion) do exert a local negative autocrine control. The higher activity of DHT compared to testosterone suggests that aromatization is not a prerequisite for androgen action. This autocrine control might explain the decrease of circulating testosterone during late fetal life [22].

Nonmammalian vertebrates.

Ho *et al.* [23] obtained an indirect evidence of a local control of testis function by estrogens in the anadromous lamprey *Petromyzon marinus*. They did not detect androgen specific binding in the testicular subcellular fractions, while definite estrogen binding activities were demonstrated both in the cytosol and nuclear extract of the testis.

Elasmobranchs show some interesting features. Although Leydig cells have been considered as estrogen target in *Squalus acanthias,* Sertoli cells are the more likely responsible for both androgen and estrogen biosynthesis than the Leydig cells [24]. Consequently, in my opinion, control of the Sertoli cells might be autocrine. Sourdaine *et al.* [25] have suggested on the basis of different sex steroid concetrations in the different zones of *Scylliorhinus canicula* testis that the germ cells must influence the steroidogenesis of Sertoli cells.

In *Torpedo ocellata* and *Torpedo marmorata,* where Leydig cells are well developed [26, 27], estradiol provokes plasma androgen increase either in intact or in hypophysectomized aminals [28]. Thus estrogen in *Torpedo* has a different effect than found in mammals. Conversely, a GnRH analog (buserelin) induces plasma androgen increase in hypophysectomized aminals in short term treatment [28] (Fig. 6) in accordance with the results available in rats (see for review, Sharpe [5]). Since irGnRH has been found in dogfish plasma [29], it remains to be established

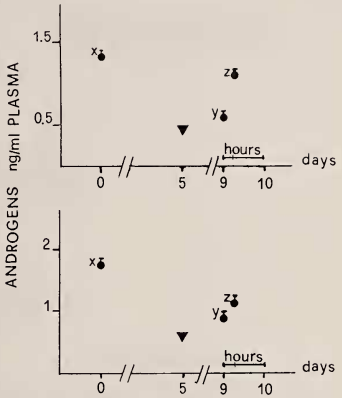


Fig. 6. Direct effect of a GnRH analog (buserelin) on the androgen production by the testis of hypophysectomized *Torpedo*. Animals were blood sampled before hypophysectomy and after 6 hr from a single intratesticular injection of buserelin. (▼) indicates the time of hypophysectomy. Androgen concentration decreases after hypophysectomy (x vs y: $p<0.01$) and increases significantly (y vs z: $p<0.01$) after buserelin treatment. The two panels are representative of two experiments (Fasano *et al.* [28]).

whether GnRH-like peptides share local regulation.

Among teleosts the steroidogenic activity of *Gobius paganellus* does not seem to be locally regulated neither by estradiol nor by GnRH analog (buserelin) (Pierantoni *et al.*, unpubl. data). In fact both substances were ineffective in inducing changes in androgen secretion. Anyway more species and hormones need to be investigated before excluding a possible local control of testicular activity in teleosts.

More thoroughly studied is the local control of testis in amphibians. Rastogi *et al.* [30] were the first to show that androgens control the formation of spermatids and play a synergistic role in enhancing the pituitary gonadotropin influence on spermatogonial proliferation in *Rana esculenta* (Fig. 7). These early data have been later interpreted as an example of local regulation [31]. Mak *et al.* [32]

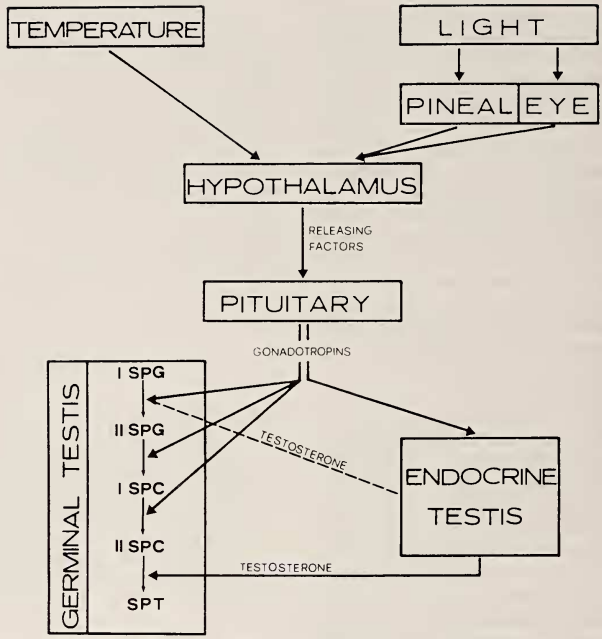


FIG. 7. A generalized scheme depicting the major relationships between the ambiental cues, hypothalamus, pituitary, pineal, eye, and endocrine tissue of the testis, involved in the control of spermatogenesis in *Rana esculenta* (Rastogi *et al.* [30]).

consider estrogens to be somehow involved in regulating the secretory activity of the Leydig cells in *Necturus* testis. Since the high level of testicular aromatase seems to be associated exclusively with the Leydig cells and estrogen binding activity is located mainly in the region of differentiating or fully differentiated Leydig cells, accordingly to my presentation, the local feedback mechanism of estrogen in *Necturus* should be considered as autocrine. Thereafter Pierantoni *et al.* [32] have shown in *Rana esculenta* that estradiol peaks at the time of the year when androgens are at baseline values. Furthermore the same research group has shown that estradiol inhibits androgen production by the testis *in vitro*, while preincubation with testosterone or DHT enhanced o-LH stimulated androgen secretion suggesting that steroids may regulate their own intratesticular levels without passing into the blood stream. The inhibitory action of estradiol in *Rana esculenta* is supported by the finding of estrogen binding activity sharing features of estrogen receptors in the testis [34].

Like mammals, *Xenopus laevis* Sertoli cells communicate with germ cells at least at specific stages of spermatogenesis [35]. The leptotene to elongating spermatid stages appear to be independent of substances produced solely by Sertoli cells. In fact spermatocytes developed *in vitro* into four spermatids as well as preleptotene spermatocytes developed into zygotene. In contrast, the develop-

ment of the spermatogonia to meiotic prophase and the transformation of spermatids into spermatozoa seem to require Sertoli cell-specific secretory products since these processes did not occur in culture.

Among nonsteroidal bioregulators, the indole amines, melatonin and serotonin, did not induce any changes in androgen production *in vitro* in *Rana esculenta* [32]. However, numerous studies *in vivo* and *in vitro* have hypothesized a local control exerted by GnRH-like material either on steroidogenesis [31, 36, 37] (Fig. 8) or on spermatogonial multiplication of the frog [38] (Fig. 9). Very recently GnRH-like material has been demonstrated in the frog testes by radioimmunoassay, immunocytochemistry [40] and HPLC purification (Cariello *et al.*, unpubl. data). The immunohistochemical staining found in Sertoli cells and in the interstitial tissue suggests multiple sites of release or uptake for GnRH-like factors. The seasonal changes in testicular irGnRH appear independent of the seasonal changes in gonadal weight and are in accordance with the androgen and spermatogenic annual profiles. In fact in *Rana esculenta* androgen production starts to increase in autumn [31, 41, 42], when irGnRH is high in the testis. The second increase of irGnRH in spring coincides with the initiation of a new wave of spermatogenesis [30]. It is interesting to note that irGnRH reaches its nadir in February when the

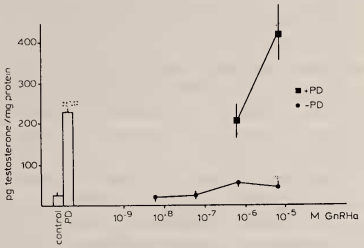


Fig. 8. Direct effect of a GnRH analog (buserelin) on the androgen production by the testis of *Rana esuculenta*. 8×10^{-7} M buserelin incubated testes show a significant increase of basal androgen output. 8×10^{-5} M buserelin induces a further significant increase in pars distalis (PD) stimulated testis (Pierantoni *et al.* [31]).

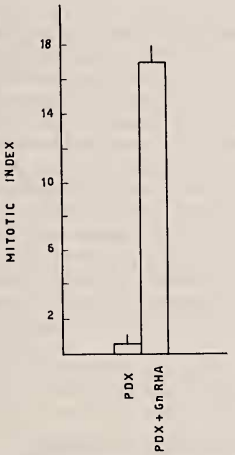


Fig. 9. Direct stimulatory effect of a GnRH analog (buserelin) on spermatogonial multiplication of hypophysectomized (PDX) *Rana esculenta* (Minucci *et al.* [38]).

mitotic index of the primary spermatogonia is at the lowest [43].

Risley *et al.* [44] have recently demonstrated that "a critical factor" in the *in vitro* maintenance of *Xenopus* spermatogenesis appears to be the preservaton of testicular organization since isolated spermatogonia and late spermatids fail to progress in either serum-supplemented or serum-free media, whereas intermediate stages of spermatogenesis continue to develop in testis fragments cultured in serum-free media. These observations strongly suggest that local regulatory factors do exist in amphibian testis.

For reptiles, we have only indirect evidence of a local control of the testicular activity. Mak *et al.* [45] have identified an estrogen-binding macromolecule in the testis of the fresh-water turtle *Chrysemys picta,* suggesting the idea that testis is an important target of estrogen action. The temporal dissociation between the plasma and testis levels of testosterone in the garter snake *Thamnophis sirtalis* strongly suggests a local control of

spermatogenesis [46].

Ciarcia and Botte [47] have shown the presence of GnRH-like material in the testis of *Podarcis s. sicula* throughout the year, except summer when testosterone is at its nadir [48]. Andò *et al.* [49] have given an indirect evidence of the inhibitory role of estradiol on androgen biosynthesis by the testis of *Podarcis s. sicula.* In fact testosterone reaches its baseline value at the time when both estradiol and progesterone reach the highest value. Furthermore the progesterone/17α-hydroxyprogesterone ratio increases indicating an inhibition of the 17α-hydroxylase activity. However it should be demonstrated whether the inhibitory effect of estradiol is direct or mediated by the pituitary.

No information is available yet for the local control of testicular activity in birds.

OVARIAN LOCAL CONTROL

The composite structure of the ovary represents another example of intriguing communications between different cell compartments. The ovarian follicle, the functional unit of the ovary, is composed of two principal cell systems, the theca and the granulosa, whose growth, development and secretion are under the endocrine control by FSH and LH. However their function is also regulated by paracrine and autocrine secretion of steroidal and nonsteroidal bioproducts.

Overview of mammals

It is well known that in mammals steroid biosynthesis by granulosa cells requires their interaction with neighbouring theca cells. Androstenedione and testosterone from theca cells cross the basal membrane, enter the granulosa layer and accumulate in follicular fluid. Granulosa cells metabolize androgens to 5α-reduced androgens, estradiol and cathecol estrogens. The androgens produced by theca cells exert important paracrine action on granulosa cell differentiation inducing the transformation of the immature granulosa cells into fully immature counterpart.

Testosterone and androstenedione are converted by the aromatizing enzymes of mature granulosa cells into estrogens which reduce the potential for direct androgenic action. On the other hand 5α-reduced androgen metabolites may act as aromatase inhibitors hence suppressing estrogen biosynthesis (see for review, Hsueh *et al.* [50]).

Local regulation of folliculogenesis and ovulation is also modulated by nonsteroidal substances like proteins, secreted by the granulosa cells (see for review, Tsafriri [51]). The list of these substances in growing rapidly. In fact candidates involved in intraovarian paracrine communications include inhibin, plasminogen activator, oocyte maturation inhibitor, relaxin, epidermal growth factor (EGF), transforming growth factors (TGFs), insulin-like growth factors (IGFs), fibroblast growth factors (FGFs), various proteases and protease/substrate inhibitors, components of the extracellular matrix such as fibronectin, oxytocin-vasopressin, opiates, GnRH-like peptides, prostaglandins, etc. However the most well known proteins secreted by the granulosa cells are inhibin, plasminogen activator, oocyte maturation inhibitor and relaxin.

Dimers of inhibin subunits synthesized by granulosa cells modulate androgen secretion by theca-interstitial cells *in vitro* [16]. $\alpha\beta$ heterodimer and $\beta\beta$ homodimer do not affect androgen biosynthesis by themselves. However when given with LH they do affect androgen biosynthesis *in vitro*: $\alpha\beta$ heterodimer further enhances LH-stimulated androgen secretion by theca-interstitial cells, whereas $\beta\beta$ homodimer suppresses LH action. When $\alpha\beta$ heterodimer and $\beta\beta$ homodimer are given together, they antagonize each other' action (Fig. 10A). Mismatching experiments show that increasing doses of $\alpha\beta$ heterodimer or $\beta\beta$ homodimer enhanced or inhibited respectively LH-stimulated androgen secretion (Fig. 10B).

Hence, similarly to the testis, also in the case of the ovary, besides the long feedback between ovarian androgen producing and pituitary LH-secreting cells, there exists an additional feedback axis between FSH-secreting and inhibin-producing cells. A paracrine control between granulosa and theca cells further mediates the cross-communications between the two axis (Fig. 11).

Plasmin, which is produced by the action of plasminogen activator, seems to intervene in de-

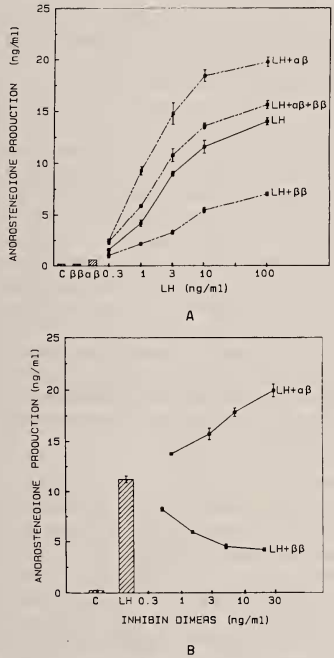


FIG. 10. Modulatory effect of inhibin dimers on LH-stimulated androgen production by cultured ovarian theca-interstitial cells of the rat. A, Modulatory effect of inhibin dimers on increasing doses of LH. B, Mismatching experiments using increasing doses of inhibin dimers (Hsueh *et al.* [17]).

creasing follicle wall strength leading to ovulation [52].

Prostaglandins, as the plasminogen activator, are also important secretory products of granulosa cells; they play a remarkable role in follicular maturation and ovulation since inhibitors of their synthesis prevent ovulation (see for review, Hsueh *et al.* [50]). Proteoglycans have been identified in the follicular fluid. They seem to be linked to the ovulation processes through increasing the viscosity of the fluid and consequently the intrafollicular pressure [53]. Relaxin, a characteristic product of the corpus luteum, is also produced by granulosa cells [54]. Too *et al.* [55] reported that relaxin stimulates granulosa cell production of plasminogen activator.

Another proposed protein produced by granulosa cells is the oocyte maturation inhibitor (OMI). OMI likely maintains the oocyte in a resting state until LH surge stimulates folliculogenesis (see for review, Hsueh [50]).

The mitogenic effect of FSH and estradiol on granulosa cells might be mediated by locally induced growth factors (EGF, IGF, etc.). In fact neither FSH nor estradiol exert mitogenic effect *in vitro*, which they do *in vivo* (see for review, Hsueh *et al.* [50]).

As regard to the intraovarian autocrine communications, Hsueh [50] has shown that estrogens stimulate granulosa cell mitoses and enhance FSH and LH action. Furthermore the still debated theory explaining how "dominant" follicles are selected to ovulate in the mammalian menstrual cycle, suggests that will be the follicle more rapidly

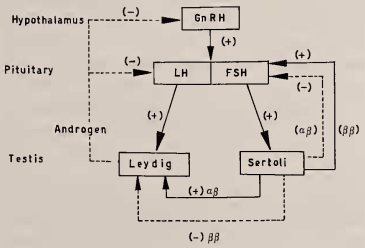


FIG. 11. Diagram of the hypothalamic-pituitary-ovarian feedback mechanisms. Based on findings of paracrine and endocrine actions of heterodimer of inhibin subunits, the cross-communication between two hypothalamic-pituitary ovarian feedback axes can be postulated. LH stimulates theca cell production of androgens, which exert negative feedback on LH release, whereas FSH stimulates granulosa cell production of inhibin, which suppresses FSH release but enhances the LH stimulation of theca cell androgen biosynthesis. In addition, LH may also stimulate granulosa cell inhibin production in mature follicles. Likewise, the $\beta\beta$ homodimer of inhibin may antagonize the action of the $\alpha\beta$ heterodimer of inhibin (Hsueh *et al.* [17]).

increasing its estrogen production to be favoured through the activation of an autocrine feedback. The selected follicles owing to their estrogen production, reduce both systemic and intrafollicular FSH, therefore the maturation of the not selected follicles fail to occur (see for review, Hsueh *et al.* [50]). They undergo atresia since they are not longer able to produce enough estrogens following FSH decline. It is not known how the selected follicles may continue to grow despite declining FSH levels (Fig. 12).

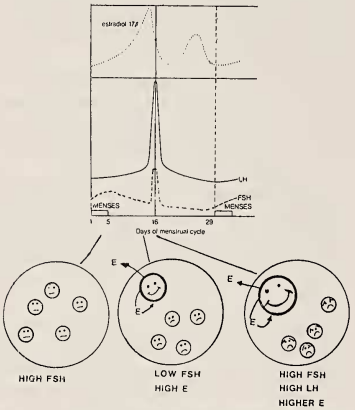


Fig. 12. Autocrine control of estrogen biosynthesis in the ovary. The "dominant follicle" is selected through the activation of an autocrine feedback (lower diagram). Upper panel, hormone profile during human menstrual cycle (Hsueh *et al.* [50]).

Autocrine control of estrogens also reflects the increased progesterone production by granulosa cells [56, 57]. Also in this case estrogens act locally enhancing the sensitivity of granulosa cells to pituitary gonadotropins.

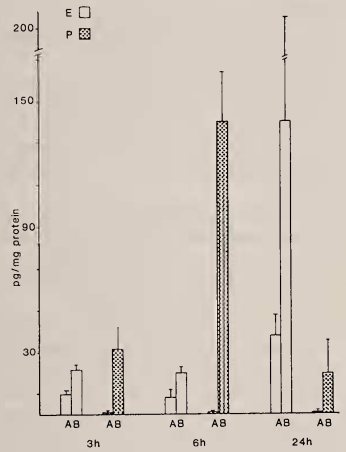
Luteal cell progesterone biosynthesis represents another clear example of an intraovarian control mechanism. Rothchild [58] has shown the stimulatory effect of progesterone on its own secretion in hypophysectomized rats. This hypothesis has been supported by the finding of specific receptors in rat granulosa cells [59, 60]. Progesterone receptors have been demostrated also in the ovary of guinea pig, rabbit, cow and human [61–63].

Ovarian GnRH deserves a particular mention, since it can act at the autocrine as well as the paracrine levels. Although the production of this peptide by the ovary has not yet been demonstrated with certainty, specific high affinity GnRH binding sites have been identified in luteal, thecal and granulosa cells at all stages of cellular differentation [64–66]. Among the multiple effects detected, I will cite both inhibition and stimulation of steroidogenesis according to the maturational stages of the follicles, the maturation of follicle-enclosed rat oocyte *in vitro,* the induction of ovulation in hypophysectomized rats, the inhibition of FSH stimulation of cAMP production, the inhibition of ovarian luteal functions in women as well as in non pregnant and pregnant rats (see for review, Hsueh *et al.,* [50]).

Nonmammalian vertebrates

No data are available for the intraovarian control of either steroidogenesis or ovogenesis in cyclostomes and elasmobranchs. In the remaining vertebrate classes the theca-granulosa communication is well known, as well as the progesterone and 17α, 20β diOH-progesterone induced maturational process of the oocyte in amphibians and teleosts (see for review, Chieffi and Pierantoni [67]). Recently in amphibians Schuetz and Lessman [68] studied the role of the follicle wall in ovulation and progesterone production in the frog *Rana pipiens.* Follicles deprived of the surface epithelium and the theca accumulated less progesterone than intact follicles. Furhermore outer surface epithelium plays some role in the pituitary-induced ovulation. In fact the "contractions" of the follicle wall are dependent upon the presence of the surface epithelium. These observations emphasize the interaction of the cellular layers of the follicle.

Estradiol exerts an important role in regulating progesterone synthesis as it has been shown by Lin and Schuetz [69, 70] in *Rana pipiens. In vitro* experiments demonstrated unequivocally that estrogen has an inhibitory effect on pituitary-induced progesterone biosynthesis and consequently on the oocyte maturation. Interesting



enough is the peak of progesterone level in the ovary when estradiol value decreases in the annual cycle of *Rana esculenta* [71]. Furthermore *in vitro* experiments show that oLH-stimulated oocytes produced estradiol which in turn inhibits progesterone biosynthesis (Fig. 13).

GnRH does not affect ovarian steroidogenesis or oocyte maturation *in vitro* in *Rana catesbeiana* and *Rana pipiens* [72]. However in *Rana esculenta* GnRH does seem to influence the ovarian function. In fact, injections of GnRH induce a signi-

FIG. 13. Modulatory effect of estradiol (E) on progesterone (P) output by cultured minced ovaries of *Rana esculenta*. Minced ovaries containing 1 mm follicles were incubated for 3, 6 and 24 hr in absence (A) and in presence (B) of 20 μg of oLH. Progesterone decreases after 24 hr incubation; concomitantly estradiol increase in oLH stimulated tubes indicating the inhibitory role of estradiol on progesterone production (Pierantoni *et al.* [71]).

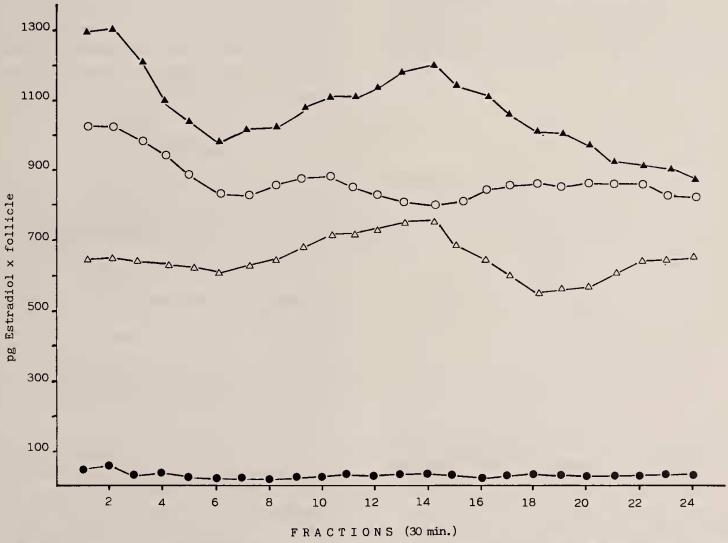


FIG. 14. Direct effect of mammalian GnRH on estradiol output by superfused ovarian follicles of *Rana esculenta*. Secretion rates of estradiol from small sized follicles (●) and continuously superfused ones with 100 ng/ml mammalian GnRH (○), homologous pituitary (▲) and mammalian GnRH plus homologous pituitary (△). Data represent averages for five females in July (Zerani *et al.* [73]).

ficant increase of plasma estradiol in hypophysectomized animals [36]. *In vitro* superfusion system confirms that GnRH acts directly on ovarian steroidogenesis as indicated by high estradiol concentrations found in the culture medium of early (~1 mm) vitellogenic follicles [73] (Fig. 14).

An inhibition of progesterone output induced by GnRH has been shown in *Rana esculenta* minced ovaries by Varriale *et al.* [74]. Discrepancies between the above quoted data may be explained either by interspecific differences or by differences in the experimental design. In fact, the size of the follicles used and the dose of peptide tested may account for discrepant results.

Sauropsida have been rather neglected so far. Among the few studies carried out, I mention the stimulation of progesterone output by the lizard *Podarcis s. sicula* follicles due to GnRH [74] and the stimulation *in vitro* by GnRH [75] (Fig. 15) and androgen [76] on progesterone production by chicken granulosa cells. It appears therefore that progesterone production may also be influenced by intraovarian steroidal and nonsteroidal environment.

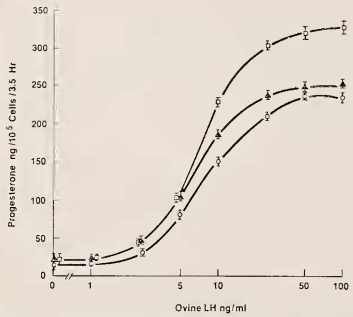


Fig. 15. Effect of GnRH on oLH induced progesterone production in granulosa cells of laying hens. Control ○; GnRH 10^{-8} △; GnRH 10^{-6} □ (Hertelendy *et al.* [75]).

CONCLUDING REMARKS

1) The science of "paracrinology" and "autocrinology" is in statu nascenti and a large volume of research is accumulating which helps in filling in the wide gaps in our knowledge of the local control of gonadal activity.
2) Although normal gonadal function ceases when gonadotropin support is withdrawn, local mechanisms, both paracrine and autocrine, subserve, mediate or modulate the action of gonadotropins according to local requirements.
3) In response to gonadotropin secretion, various gonadal compartments interact in highly integrated manner not only to modulate steroid secretion, but also to ensure the regular succession of gametogenic events.
4) We know for sure that gonadal activity is responsive to environmental and neuroendocrine factors, but only now we start to consider that these factors need for their goal also paracrine and autocrine regulation (Fig. 16).
5) Although the majority of the research in this field deals with mammals, the growing literature shows that not only local regulation occurs in nonmammalian vertebrates, but also that different mechanisms might modulate locally gonadal activity.

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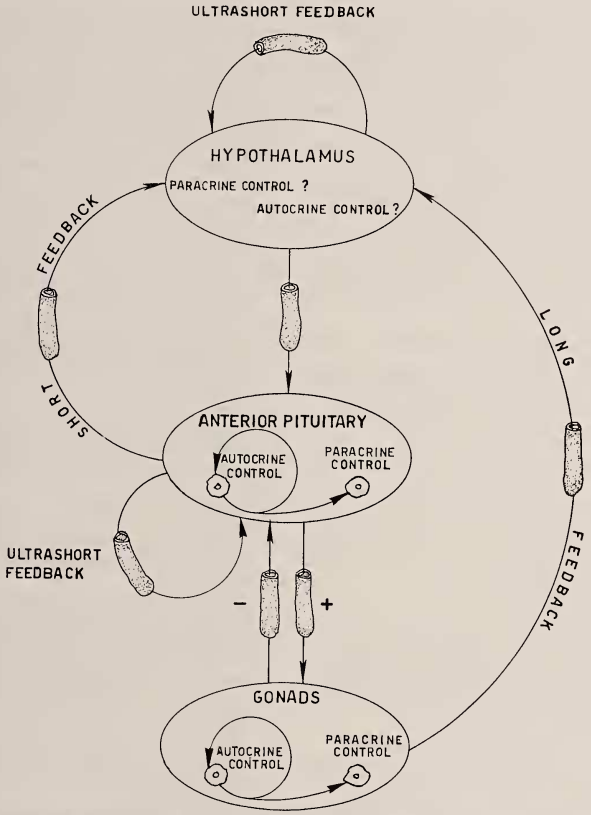

FIG. 16. The up-to-date scheme of the endogenous mechanisms modulating gonadal activity.

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